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# Whey Utilization

## III. Oxygen Absorption Rates and the Growth of *Saccharomyces fragilis* in Several Propagators

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Maximal growth of aerobic organisms in mass culture is dependent on an adequate supply of oxygen. Since microorganisms freely use oxygen in quantities consistent with their metabolic capabilities, the availability of oxygen in the dissolved state can be a limiting factor.

Oxygen is sparingly soluble in water and in the solutions commonly used for culture media, and mechanical devices are generally required to accelerate the solution of the gas in the liquid. The kinetics of this system have been studied extensively (Hixon and Gaden, 1950; Bartholomew *et al.*, 1950) and formulas have been derived to determine the rates of solution of oxygen. Thus, the oxygen absorption rate (OAR) may be obtained by the method of Cooper, Fernstrom, and Miller (1944), or the oxygen absorption coefficient ($K_LA$) may be determined by polarographic techniques (Eckenfelder, 1957). These values are a characteristic of the mechanical devices involved and are constant for a set of described conditions. Variations in the conditions (air flow, rate of agitator speed, depth of liquid, position of devices) may result in large changes in the OAR of a mass culture device.

Successful mass culture of microorganisms may be facilitated by knowledge of the peak oxygen demand of the organisms. A propagator with an OAR great enough to assure an adequate supply of dissolved oxygen can then be designed. *Saccharomyces fragilis*, growing in a cheese whey medium, consumed 110 ml oxygen per L medium per min at peak demand (Wasserman, 1960). To assure comparable growth of the yeast in the whey medium in another propagation device, it is necessary that the propagator dissolve at least 110 ml oxygen per

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L medium per min (or 5 mM oxygen per L per min). Growth of *S. fragilis* in propagators with various oxygen dissolving devices is described in this report.

## Materials and Methods

The yeast, *S. fragilis*, was grown as previously described (Wasserman, 1960) in whey containing 0.5 per cent $(NH_4)_2SO_4$, 0.5 per cent $K_2HPO_4$, and 0.1 per cent yeast extract. The pH and temperature conditions were the same as in the previous paper (Wasserman, 1960). Fifteen-L quantities were used in two propagators and 24 L of medium were used in the third, a Waldhof[2, 3, 4] propagator. The first two propagators were glass jars 12 in. in diameter and 24 in. high with total capacities of 40 L each. In propagator no. 1, agitation was achieved with a turbine impeller (figure 1 (*A*)) powered by a ⅛ HP Lightnin motor. Air was introduced through a tube led from the top plate and centered as closely as possible under the turbine blades.

Propagator no. 2 was of similar dimensional design. Agitation was carried out with a ½ HP Rheinhütte[5] motor and the agitator shown in figure 1(*B*). The aerator tube was placed immediately beneath the center opening of the agitator. Originally designed as a mixin device, the impeller draws liquid through the top and bottom openings, and expels it rapidly from the side vents while turning. The air, sucked in the bottom opening, is broken up into many small bubbles by the strong shearing action.

The third propagator, the laboratory size Waldhof, is in successful industrial operation in this country and in Europe. A number of papers describing the design and use of this apparatus have been published (Saeman, 1947; Demmler, 1950). Essentially designed for continuous culture operation, the Waldhof can be used for batch process propagation. The apparatus (figure 2) consists of a steel cylindrical tank, open at the top, 15 in. in diameter and 24 in. high. There is an inner open draft tube supported above a turbine-like impeller.

Yeast dry weight was determined by centrifuging a 5-ml aliquot of the culture liquid, washing with distilled water, and drying overnight at 105 C. Weights were corrected for the weight of seed yeast.

Lactose was determined by the method of Stiles, Peterson, and Fred (1926). A Beckman Oxygen Analyzer, model C,[6] was used to determine the residual oxygen content of the effluent air from the propagator. The quantity of oxygen used by the organisms was computed as described by Wasserman (1960).

Oxygen absorption rates (OAR) of the propagators were determined by the use of the sulfite method of Cooper, Fernstrom, and Miller (1944) as modified by Corman *et al.* (1957). Results are expressed as mM oxygen per L medium per min.

[2] It is not implied that the U. S. Department of Agriculture recommends the companies named or their products to the possible exclusion of others in the same business.

[3] Stainless Steel Products Co., St. Paul, Minnesota.

[4] Obtained through the courtesy of Dr. A. J. Wiley, Sulfite Pulp Manufacturers' Research League, Appleton, Wisconsin.

[5] The Rheinhütte motor and aerator-agitator device were made available through the courtesy of Neumann and Weaver, Inc., Fairlawn, New Jersey.

[6] Beckman Instruments, Inc., Fullerton, California.

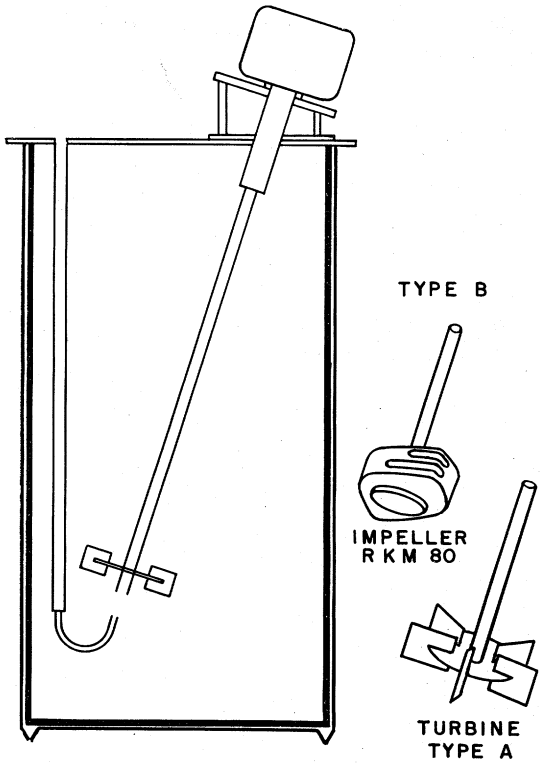


*Figure 1.* Schematic sketch of the 15-L propagator used for growing *Saccharomyces fragilis*: type A—turbine impeller; type B—Rheinhütte impeller.

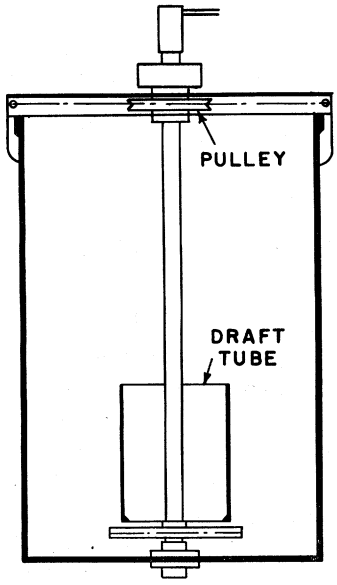


*Figure 2.* Schematic sketch of the Waldhof propagator.

## Results

The oxygen dissolving characteristics of the propagators were first determined. Increasing the quantity of air passing through propagator no. 1 resulted in an increase in the volume of oxygen dissolved (figure 3 (*A*)). This was true only when the turbine agitator was used. A propeller-type agitator was substituted for the turbine impeller, and all other factors were kept the same. Visual observation of the action of the propeller-type impeller showed excellent turbulence of the liquid with fine break-up of the air bubbles. However, increasing the air flow through the propagator had no effect on the solution of oxygen into the liquid (figure 3 (*B*)).

The effect of increasing the air flow in propagator no. 2, containing the Rheinhütte aeration-agitation system, is shown in figure 3 (*C*). A maximal OAR of 3.4 mM oxygen per L per min was reached at an air flow of 37.5 L per min. This rate of air flow was the limit of the Fischer Flowmeter[7] used with this apparatus. A further check with a roughly calibrated flowmeter of greater capacity showed no increase in OAR with an air flow of approximately 60 L per min. In a previous paper (Wasserman, 1960), *S. fragilis* was grown in 500-ml quantities in a small propagator. Changes in the OAR of this apparatus as the air flow was varied are shown in figure 3 (*D*).

Changes in the oxygen absorption rate in propagator nos. 1 and 2 could be effected only by variation of the air flow, as the motors operated at a single rate of rotation. The Waldhof propagator, on the other hand, had a belt drive, and by appropriate connection of pulleys, the agitator speed was varied as well as the air rate. The OAR values attained by these changes are shown in figure 4. Oxygen absorption rate is a function of the rate of agitation as well as air rate.

The peak oxygen demand of growing *S. fragilis*, cultured under the conditions described, was approximately 100 ml per L per min, or 5 mM oxygen per L per min (Wasserman, 1960). To assure an adequate oxygen supply to satisfy this demand, propagator no. 1 should be aerated at 30 L per min. Propagator no. 2, with a maximal OAR of 3.4 mM oxygen per L per min, would not provide the oxygen necessary for maximal growth and it was expected that a lower yield of yeast would be obtained. In the Waldhof propagator, the 5 mM oxygen per L per min could be attained with an air rate of approximately 26 L per min at 1,500 rpm agitator speed, and 45 L per min at 1,150 rpm. At 850 rpm agitation, the maximal OAR was approximately 3.4, or less than the rate required for maximal growth.

Yeast cultures were grown in the propagators to determine the effect of the several aerator-agitator designs on yeast yields. Propagator nos. 1 and 2 were compared directly. The required quantity of yeast (380 g dry weight) was suspended in 30 L of the whey medium, 15-L quantities were placed in each of the propagators, and air at 30 L per min supplied to both. The oxygen consumption patterns, yeast yields, and lactose utilization are shown in figure 5. Yeast growing in propagator no. 1, the turbine-agitated apparatus, had the highest

[7] Fischer and Porter Company, Hatboro, Pennsylvania.

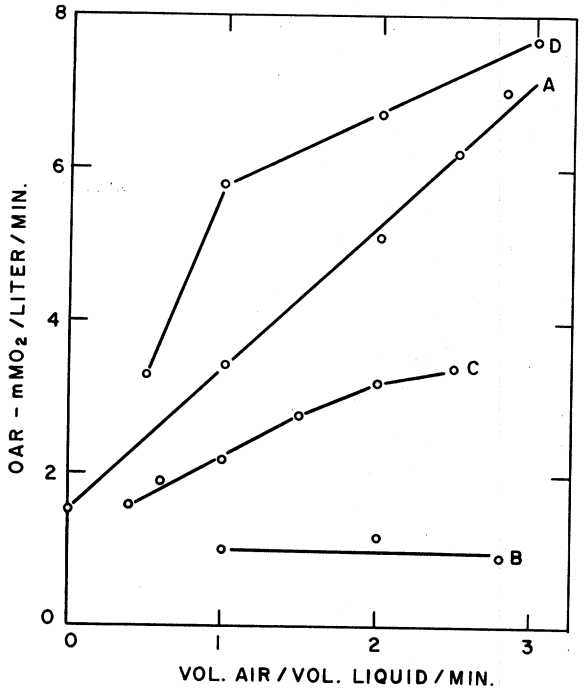


*Figure 3.* Oxygen absorption rates (OAR) achieved by the use of different impellers and propagators as a function of the ratio: air volume:liquid volume. Fifteen L liquid in propagator (see figure 1): *A*, turbine-type impeller; *B*, propeller-type impeller; *C*, Rheinhütte impeller; *D*, 500 ml liquid in small propagator (Wasserman, 1960).

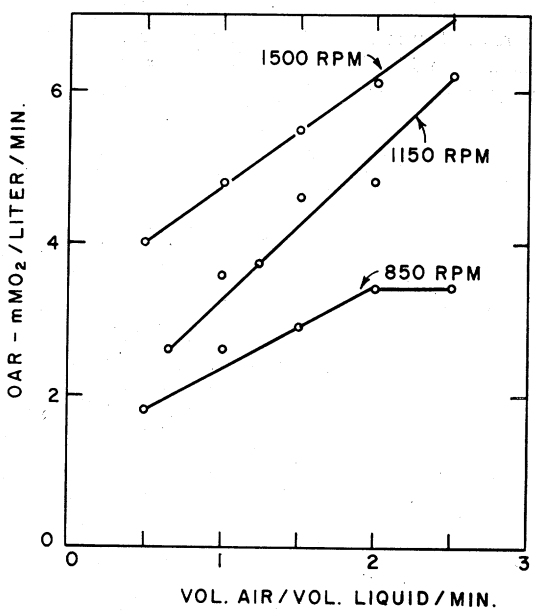


*Figure 4.* Oxygen absorption rates (OAR) achieved in the Waldhof propagator by varying the speed of agitation and the ratio: air volume:liquid volume.

yield, 24.7 g dry yeast per L medium, as opposed to 15.2 g per L for propagator no. 2. (The 24.7 g per L is 91 per cent of the maximal yield of yeast theoretically obtainable from whey medium (Wasserman *et al.*, 1958).) Lactose utilization was more rapid in propagator no. 2. At the end of 3 hr, 33 per cent of the lactose in propagator no. 1 was still present, whereas the residual sugar in propagator no. 2 was 11 per cent of the initial value. In both propagators, sugar was usually completely consumed within 4 hr.

Yeast cultured in the Waldhof propagator, however, grew well even under presumably adverse conditions of oxygen solubility. Operating the Waldhof at an agitator speed of 1,500 rpm and an air flow of 24 L per min assures the desired OAR of 5 mM oxygen per L per min. This rate was also achieved by reducing the agitator speed to 1,150 rpm, but increasing aeration to approximately 48 L per min. At an agitator speed of 1,150 rpm and 33 L per min aeration, the OAR dropped to 4.0 mM oxygen per L per min, whereas at the same speed but 24 L per min air flow, the OAR was only 3.4 (figure 4). Yeast was grown in the Waldhof propagator under the agitator and air flow conditions described above. Direct observation of the culture was necessary so the top of the propagator was not used. Collection of the effluent air for oxygen analysis was not attempted, therefore the oxygen consumption of yeast grown under the various conditions is not known. Although the net dry weight of the yeast yeilds was similar in all the propagations, there were differences in the rate of sugar utilization (figure 6).

There appeared to be no relation between the OAR values and the growth characteristics of the yeast in the Waldhof apparatus, as opposed to the results obtained in the other propagators. The lack of correlation is difficult to explain. It is possible that the rate of oxygen solution in the culture medium is greater than the OAR in the sulfite solution of the method of Cooper, Fernstrom, and Miller. Pirt and Callow (1958), on the other hand, found that the maximal oxygen solution

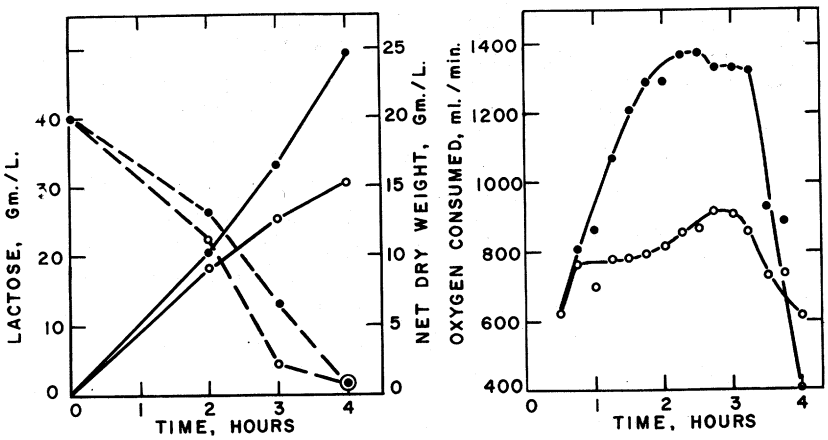


*Figure 5.* Comparison of the lactose utilization, net yeast yield, and oxygen consumption of *Saccharomyces fragilis* growing in the 15-L propagator stirred with a turbine-type impeller (●——●), or the Rheinhütte impeller (○——○). Rate of aeration: 30 L per min for both propagations.

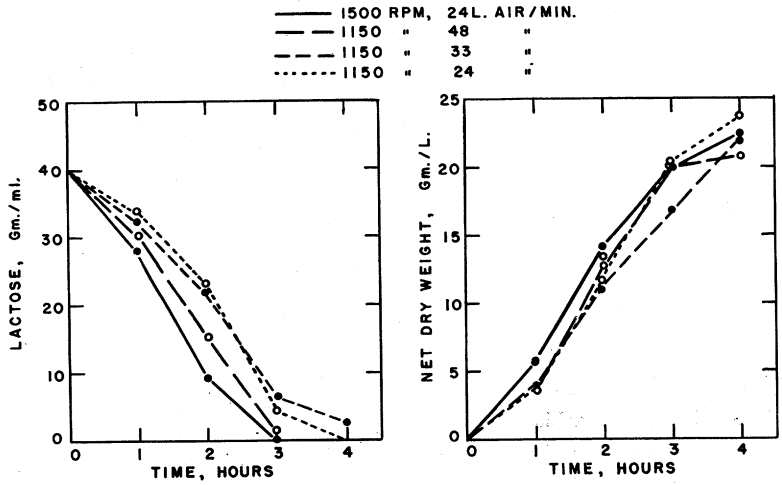


*Figure 6.* Comparison of the lactose utilization and net yeast yield of *Saccharomyces fragilis* growing in the Waldhof propagator under varying conditions of air flow and agitation.

rates in bacterial cultures were 0.5 to 0.7 times those in sulfite solution with a particular propagator of conventional construction. The Waldhof propagator, however, has unusual operating characteristics that could result in an increase in OAR on substituting medium for sulfite solution. Agitation and aeration that cause no change in the sulfite solution create large quantities of foam with whey medium. Even under control of an antifoam agent, quantities of air may be entrapped in the foam layer. The foam falling into the draft tube comes into violent contact with the agitator-aerator at the bottom. The medium may thus absorb more oxygen than the nonfoaming sulfite solution.

Good growth with low aeration rates also has been reported for the Waldhof apparatus with yeast grown in spent wood sulfite liquor (Dr. A. Wiley, personal communication).

## Summary

*Saccharomyces fragilis* was grown in whey medium in three propagators of varying aerator-agitator designs. The oxygen absorption rates (OAR) of these propagators were dependent on the agitator design and speed, and the aeration rate.

Propagator characteristics may be selected to provide a sufficient supply of dissolved oxygen when the oxygen demand of the yeast culture under given conditions is known. The yeast yield and oxygen consumption during growth are related to the OAR of a propagator in conventional apparatus. In the Waldhof propagator, good yeast yields were obtained in media presumably containing less dissolved oxygen.

## References

Bartholomew, W. H., Karow, E. O., Sfat, M. R., and Wilhelm, R. H. 1950 Oxygen transfer and agitation in submerged fermentations. Mass transfer of oxygen in submerged fermentation of *Streptomyces griseus*. Ind. Eng. Chem., **42,** 1801–1809.

Cooper, C. M., Fernstrom, G. A., and Miller, S. A. 1944 Performance of agitated gas-liquid contractors. Ind. Eng. Chem., **36,** 504–509.

Corman, J., Tsuchiya, H. M., Koepsell, H. J., Benedict, R. G., Kelley, S. E., Feger, V. H., Dworschack, R. G., and Jackson, R. W. 1957 Oxygen absorption rates in laboratory and pilot plant equipment. Appl. Microbiol., **5,** 313–318.

Demmler, G. 1950 Yeast culture on whey by the Waldhof process. Milchwissenschaft, **14,** 11–17.

Eckenfelder, W. W., Jr. 1957 Polarographic measurement of dissolved oxygen. Symposium on determination of dissolved oxygen in water. Am. Soc. Testing Materials, Spec. Tech. Publ. No. 219, 18–29.

Hixon, A. W. and Gaden, E. L., Jr. 1950 Oxygen transfer in submerged media. Ind. Eng. Chem., **42,** 1792–1801.

Pirt, S. J. and Callow, D. S. 1958 The relationship between maximum oxygen solution in a bacterial culture and in a sodium sulfite solution under comparable aeration conditions. J. Appl. Bacteriol., **21,** 206–210.

Saeman, J. F. 1947 Aerobic fermentor with good foam control properties. Anal. Chem., **19,** 913–915.

Stiles, H. R., Peterson, W. H., and Fred, B. E. 1926 A rapid method for the determination of sugar in bacterial cultures. J. Bacteriol., **12,** 427–439.

Wasserman, A. E., Hopkins, W. J., and Porges, N. 1958 Whey utilization. Growth conditions for *Saccharomyces fragilis*. Sewage and Ind. Wastes, **30,** 913–920.

Wasserman, A. E. 1960 Whey utilization. II. Oxygen requirements of *Saccharomyces fragilis* growing in whey medium. Appl. Microbiol., **8,** 291–293.